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RADIOPROTECTIVE AGENTS

A Correlative Study of RNA Content and the Degree of Radioresistance of Cells in Vitro

TECHNICAL DOCUMENTARY REPORT NO. SAM-TDR-63-17

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USAF School of Aerospace Medicine
Aerospace Medical Division (AFSC)
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FOREWORD

This report was prepared by the following personnel of the Pasadena Foundation for Medical Research, Pasadena, California:

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WILLIAM C. SLICK, B.S.

ABSTRACT

In vitro studies revealed that a 24-hour preirradiation treatment of an amnion cell line with yeast RNA increased cell survival in proportion to the yeast RNA content in the medium. Conversely, ribonuclease treatment increased radiosensitivity. However, the protective response was observed only after 8 to 10 hours of preirradiation treatment with yeast RNA. Some increase in cell survival was recorded at 4 to 6 hours when an alkaline hydrolysate of yeast RNA was used.

Microspectrophotometric measurements of cytoplasmic basophilia revealed substantially increased optical density within 4 hours of incubation with RNA. The delay in a protective response during this period may have been due to the time required for digestion and resynthesis into an active radioprotective agent. A correlation between RNA synthesis and the radioprotective response confirmed this hypothesis.

This technical documentary report has been reviewed and is approved.



ROBERT B. PAYNE
Colonel, USAF, MSC
Chief, Operations Division

A CORRELATIVE STUDY OF RNA CONTENT AND THE DEGREE OF RADIORESISTANCE OF CELLS IN VITRO

1. INTRODUCTION

Previous reports (1, 2) have indicated that the amount of ribonucleic acid (RNA) added to culture medium prior to 700 r gamma radiation increases the survival rate of amnion cells in proportion to the RNA content of the media to a maximum of 0.2 mg./ml. Recently this finding was extended by the observation that depolymerized ribonucleic acid had a similar effect with maximum protection being achieved at 0.3 mg./ml. (3). These data suggest that within certain limits the radioprotective response of the cells in vitro can be correlated with quantitative amounts of yeast RNA in the medium, and presumably within the cell. It was observed, however, that the protective response occurred only after 8 to 10 hours of preirradiation treatment with yeast RNA and after 4 to 6 hours' preirradiation treatment with depolymerized RNA. If yeast RNA acts as a radioprotective agent when ingested, one might predict that the intracellular RNA content was being modified only after 4 to 8 hours. To test this hypothesis, an attempt was made to measure intracellular RNA content and correlate such levels with the degree of radioresistance of cells in vitro.

2. SUMMARY

In vitro studies revealed that a 24-hour preirradiation treatment of an amnion cell line with yeast RNA increased cell survival in proportion to the amount of yeast RNA added to the medium. Conversely, ribonuclease resulted in an increased radiosensitivity in this system. When the preirradiation treatment period was varied from 4 to 24 hours, the protective response was observed only after 8 to 10 hours with yeast RNA. Some increase in cell survival

was recorded at 4 to 6 hours when an alkaline hydrolysate of yeast RNA was used.

Microspectrophotometric measurements of cytoplasmic basophilia suggested that yeast RNA was ingested by the cells in substantial amounts within 4 hours. The delay in a protective response during this period may have been due to the time required for digestion and resynthesis into an active radioprotective agent. A strong correlation between RNA synthesis (as seen during depolymerized RNA ingestion) and the radioprotective response seemed to confirm this hypothesis.

3. MATERIALS AND METHODS

Duration of RNA treatment

The Fernandes line of amnion cells was used as the test object for all experiments. A total of 64 T-30 flasks were seeded with 250,000 such cells per flask. After a 24-hour incubation period one-half of the cultures were treated with 0.1 mg./ml. yeast RNA for periods ranging from 4 to 24 hours. An equal number of control flasks were kept for equivalent periods of time with control medium. After treatment, medium was removed from all flasks and the cells were irradiated with 700 r gamma radiation in an air phase. Fresh control medium was then added and the cultures were incubated for 4 days. Cells from each flask were then trypsinized, washed, and suspended in saline, and enumerated with the aid of a Coulter electronic cell counter. The ratio of the number of surviving cells in treated cultures to the population of control flasks was calculated. A similar procedure was followed for cells given a preirradiation treatment with 0.2 mg./ml. depolymerized RNA (obtained from an alkaline

hydrolysis of commercial yeast RNA as described in an earlier report (3)). The treatment periods with this substance, prior to irradiation, ranged from 2 to 24 hours. An additional parameter was examined by treating 10 flasks of amnion cells for a 2-hour period with depolymerized RNA, followed by an exchange with normal Eagle's medium, and by incubating for a period of 4 hours prior to 700 r gamma radiation. An additional 10 flasks served as controls for this experiment.

Treatment with ribonuclease

Fifteen T-30 flasks were seeded with 250,000 amnion cells per flask. Five of these flasks were treated with 0.5 mg./ml. commercial ribonuclease for a 1-hour interval, 5 were treated with the same concentration of ribonuclease for a 2-hour period, and the remaining 5 served as controls. After incubation in ribonuclease, all experimental and control cells were subjected to 700 r gamma radiation in an air phase. The medium was changed and the flasks were incubated for 4 days. The cells from each flask were then harvested by trypsinization, washed, suspended in saline, and enumerated with the aid of the Coulter cell counter.

Estimation of intracellular RNA content

Amnion cell populations, growing on flying coverslips in roller tubes, were treated for varying periods ranging from 0 to 10 hours, with either 0.1 mg./ml. yeast RNA or 0.2 mg./ml. depolymerized RNA dissolved in Eagle's medium. Following the incubation period, the cells were fixed and stained with methyl green-pyronine as described by Brachet (4). The basophilia of the cytoplasm of cells in each culture was estimated with the microspectrophotometric technic as modified by Rounds and Pomerat (5), using a wavelength of 565 m μ . The optical density of cytoplasmic basophilia, less the optical density of ribonuclease-treated control cells, was considered a relative estimation of the cytoplasmic RNA content.

4. RESULTS

The radiosensitivity of amnion cells after treatment with either yeast ribonucleic acid or ribonuclease appeared to be correlated with the amount of intracellular RNA (table I). The response of cells treated in a range from 0.01 to 0.1 mg./ml. yeast RNA showed an 11% to a 79% increase in the surviving cell population as compared to control cultures. Consistent with this trend was the finding that ribonuclease-treated amnion cells appeared to show an increase in radiosensitivity after 1- or 2-hour treatments. The data suggested that approximately 20% fewer cells survived 700 r gamma radiation when treated with ribonuclease.

TABLE I

Surviving human amnion cell population 4 days after 700 r gamma radiation

Agent	Duration of treatment (hours)	Dose (mg./ml.)	Treated/control ratio
Yeast RNA	24	0.1	1.79
Yeast RNA	24	0.05	1.43
Yeast RNA	24	0.01	1.11
Control	—	—	1.00
Ribonuclease	1	0.5	0.81
Ribonuclease	2	0.5	0.77

Surviving cell populations which were incubated for varying periods of time in the presence of yeast RNA prior to 700 r gamma radiation indicated a variable response: the 4-hour treatment produced an increased sensitivity, and 8-hour periods or longer resulted in increased resistance to gamma radiation (fig. 1). In comparison, the populations which survived radiation, following incubation with the hydrolyzed RNA, showed an increase in radioresistance at 4- and 6-hour treatment periods, with a maximum apparently occurring at 12 hours. Cells exposed to the depolymerized RNA for 2 hours, then irradiated after an additional 4 hours' incubation in control medium, showed approximately the same response as cells exposed for a full 6-hour period (table II).

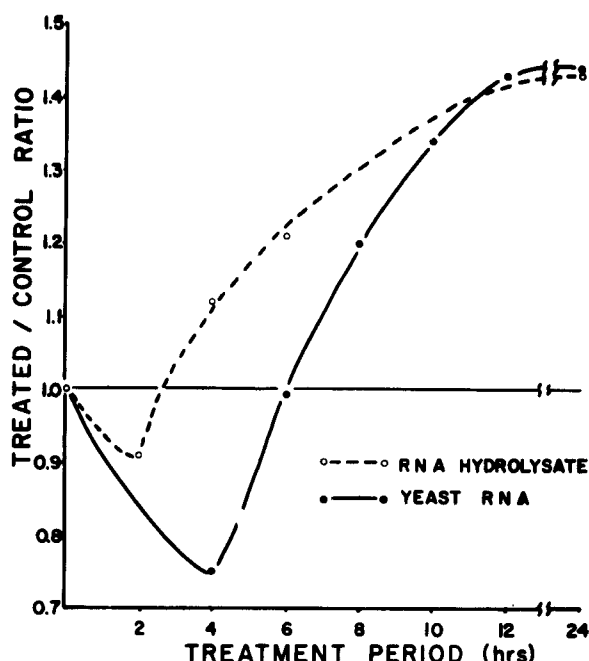


FIGURE 1

The response of amnion cells to 700 r gamma radiation. Each point represents the treated/control ratio of surviving cell populations derived from counts of 5 to 10 flasks each of experimental and control cultures.

TABLE II

The response of amnion cells to 700 r gamma radiation following incubation in 0.2 mg./ml. depolymerized RNA

Preirradiation treatment period (hours)	Treated/control ratio of surviving cell numbers
0	1.00
2	0.91
4	1.12
6	1.21
2/6*	1.19
8	1.28
12	1.44
24	1.43

*Cells were treated with the RNA hydrolysate for the first 2 hours of a 6-hour incubation period.

Optical density measurements of cells incubated in the presence of yeast RNA indicated a rapid uptake of this material into the cytoplasm, reaching a maximum at approximately 4 to 6 hours of incubation (fig. 2).

When the radioresponse rate of the cell population to gamma radiation was superimposed on the rate of RNA uptake, it was observed that the elevated cytoplasmic RNA content at the 4-hour interval was associated with an increase in radiosensitivity. As the rate of RNA ingestion decreased, the radioresistance began to increase, reaching a maximum at 12 hours of incubation.

The estimated amount of cytoplasmic basophilia, following incubation with the depolymerized RNA, indicated that the oligonucleotides and polynucleotides were ingested by the cell within a 1-hour period of incubation. Following this initial increase in stainability, the 2- and 3-hour intervals indicated an average optical density equivalent to control values, suggesting degradation to mononucleotides. The apparent synthesis of a basophilic substance was associated with an increase in the staining reaction from the 3d to 10th hour of

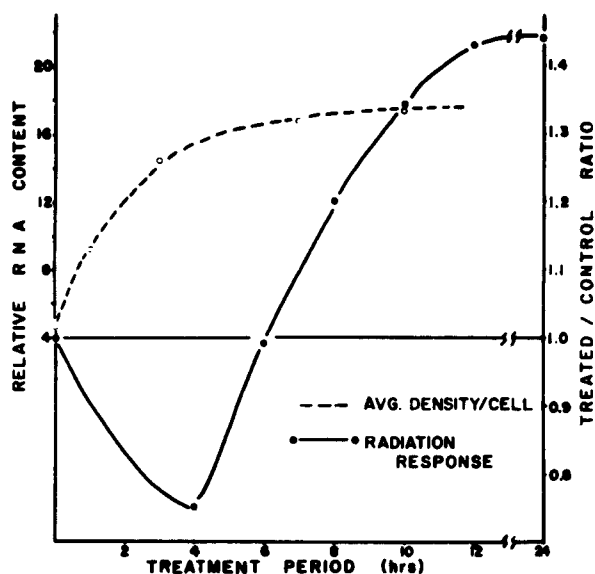


FIGURE 2

The relative RNA content, in arbitrary units, of amnion cells incubated in 0.1 mg./ml. yeast RNA for varying periods of time (broken line). Superimposed on the same time scale is the radiation response of yeast RNA-treated cells taken from figure 1 (solid line).

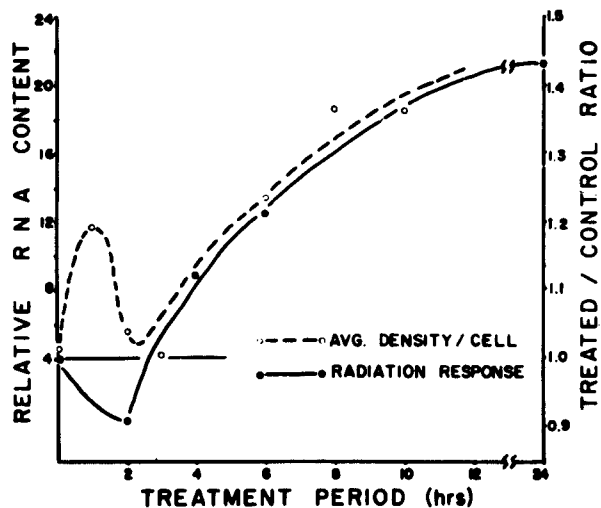


FIGURE 3

The relative RNA content, in arbitrary units, of amnion cells incubated in 0.2 mg./ml. depolymerized RNA for varying periods of time (broken line). Superimposed on the same time scale is the radiation response of depolymerized RNA-treated cells taken from figure 1 (solid line).

incubation in the depolymerized RNA (fig. 3). The radioresponse of amnion cells following similar treatment intervals indicated that a minimal radioprotective response occurred after 2 hours of incubation, followed by a rapid increase in radioresistance between 2 and 12 hours (fig. 3). The rate of increase of radioresistance showed a correlation with the rate of increased basophilia of the cytoplasm from 3- to 10-hour intervals.

5. DISCUSSION

The work by Schwarz and Rieke (6) indicates that malignant cells can ingest macromolecular RNA. In addition, Borenfreund and Bendich (7) have indicated that polymerized nucleic acid (DNA) may be incorporated intact into HeLa cells within a 2-hour period. These reports suggest the possibility that HeLa and KB cells may incorporate the polymerized yeast RNA as well as amnion cells. Rounds (1) has reported, however, that HeLa and KB cell lines treated with 0.1 mg./ml. yeast RNA for 24 hours prior to 700 r gamma radiation show no protective response, in contrast to amnion and other established lines. This differential action of yeast RNA rules out the likelihood that the nucleic acid acts as a radical scavenger or that it increases the number of radiosensitive targets.

The data in table I suggested that the radioresponse of amnion cells was correlated with

the amount of RNA added to the medium. In spite of the high levels of cytoplasmic RNA following 4 hours of incubation in RNA-supplemented medium, however, the survival rates following radiation indicated an increased sensitivity (fig. 2).

Cell populations treated with depolymerized yeast RNA for 2 hours appeared to enhance radiation damage. When the 2-hour treatment was followed by 4 hours of incubation in normal medium, however, the cell cultures showed an increased survival rate as compared with controls (table II).

The lack of response after a 24-hour treatment of HeLa and KB cells or after 4 hours' treatment of amnion cells indicated that yeast RNA did not, in itself, effect a decrease in the degree of radiation damage. It is possible that amnion cells hydrolyzed the polymerized nucleic acid (or the oligonucleotides) to smaller molecular weight substances, then synthesized basophilic compounds from the degraded starting materials. This hypothesis appeared to be confirmed by changes in the optical density of cells incubated in medium enriched with depolymerized RNA (fig. 3). The increase in cytoplasmic basophilia, which occurred between 3 and 10 hours of incubation, suggested the rate of synthesis of amnion RNA. The correlation of the increase in cellular radioresistance with this increase in basophilia implicated the requirement for a specific RNA to modify the radioresponse of the cells in culture.

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USAF School of Aerospace Medicine, Brooks AF Base, Tex.

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3. Radiation protection
- I. AFSC Task 775702
- II. Rounds, D. E., and Slick, W. C.
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